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# The Effects of 22-kHz Ultrasonic Vocalization Duration on Defensive Behavior in Rats

Christian Hinderer

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**Christian Hinderer and Andrew J. Niemiec**
**Neuroscience Program**
**Kenyon College, Gambier Ohio**

## Introduction

- Adult rats produce ultrasonic vocalizations (USVs) at two frequencies: 22 kHz and 50 kHz.
- The 22 kHz vocalizations are believed to serve a role as an antipredator warning to other rats (Blanchard et al., 1992).
- In order for these 22 kHz USVs to function as a form of communication, there must be some aspect of the calls that serves to encode and convey information.
- The frequency content of the calls shows little variation, so it seems that call duration may be more important to the USV's meaning (Brudzynski, 2005).
- This experiment examines the effects of USV duration on the exploratory behavior of rats in an open field test.
- The experiment will also examine how the sexes differ in their responses to 22 kHz USVs.

## Methods

- 22 kHz USVs were elicited from three adult male rats using predator odor.
- Vocalizations were recorded using a bat detector and ultrasound processor, then stored and manipulated on a computer.
- Recordings were split into individual calls and recombined to form three call groups containing USVs of similar duration (short, medium or long duration).
- For each call duration, 10 male and 10 female rats were individually tested in an open field apparatus containing two hiding places. The apparatus also contained 16 pieces of Fruit Loops distributed in the open areas to encourage exploratory behavior.
- Each animal was presented with 5 repetitions of a specific call group (either short, medium, or long duration) at the rate of one call/minute.
- 4 male and 4 female rats also served as "no-call" controls.

## All Animals

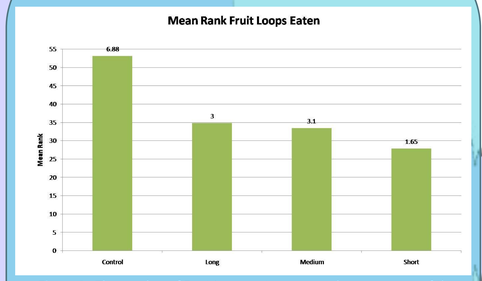


**Figure 1.** The number of Fruit Loops eaten was used as a measure of the animal's level of comfort in its environment. The group receiving short calls ate the fewest Fruit Loops, indicating greater alarm. Data were analyzed using a nonparametric Kruskal-Wallis one-way ANOVA, as the homogeneity of variance assumption for parametric statistics was not met. There was an overall significant effect of call duration ($X^2 = 9.44$, df = 3, $p < 0.024$). A Mann-Whitney U Test (with Bonferroni correction for multiple comparisons) showed a significant difference between the short call and control group ($U = 142.5$, $z = 3.23$, $p < 0.0012$). Mean number of Fruit Loops eaten appear above bars.

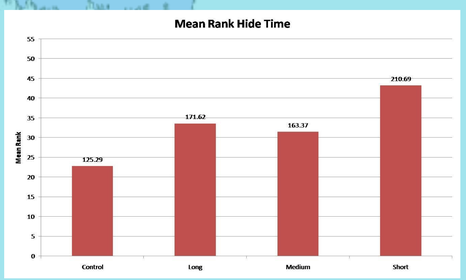


**Figure 2.** Hide time reflects the total time an animal spends inside the available enclosures. Data were analyzed using a nonparametric Kruskal-Wallis one-way ANOVA, as homogeneity of variance assumption for parametric statistics was not met. A clear trend is visible, again indicating higher levels of defensiveness among the short call group, but the effect of call duration was not significant ($X^2 = 7.22$, df = 3, $p < 0.0653$). Means (seconds) appear above bars.

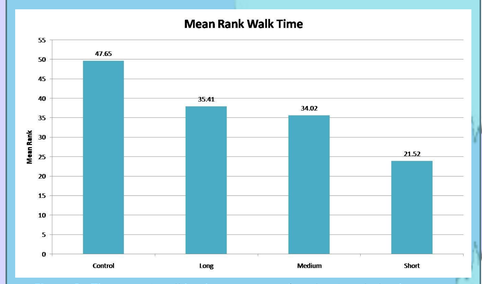


**Figure 3.** Time spent walking is a measure of exploratory behavior, an indicator of comfort with the environment. Data were analyzed using a nonparametric Kruskal-Wallis one-way ANOVA, as homogeneity of variance assumption for parametric statistics was not met. There was a significant effect of call length ($X^2 = 11.1$, df = 3, $p < 0.0112$). A Mann-Whitney U Test (with Bonferroni correction for multiple comparisons) showed a significant difference between the short call and control group ($U = 140$, $z = 3.10$, $p < 0.0019$). Means (seconds) appear above bars.

## Males Only

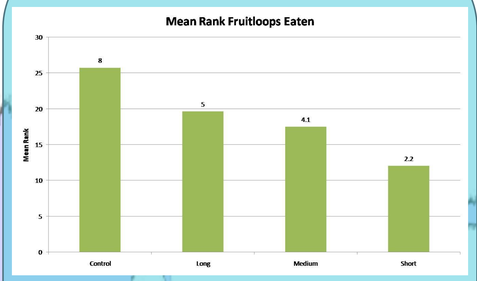


**Figure 4.** The males who were presented with a call ate fewer Fruit Loops than those who received no call. The effect was strongest with calls of shorter duration. The decrease in food seeking behavior indicates that the shorter calls were perceived as indicating a greater level of threat. A Kruskal-Wallis one-way ANOVA did not show a statistically significant effect of call duration ($X^2 = 6.21$, df = 3, $p < 0.102$). Means (number of Fruit Loops eaten) appear above bars.

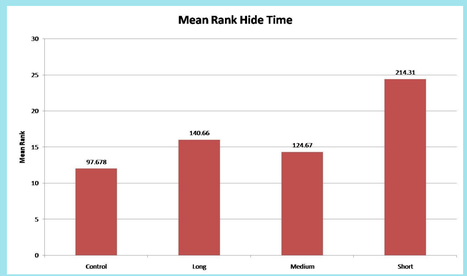


**Figure 5.** Males tended to spend more time hiding in the short call condition than any of the other conditions, indicating a greater degree of alarm. A Kruskal-Wallis one-way ANOVA did not show a statistically significant effect of call duration ($X^2 = 7.28$, df = 3, $p < 0.0635$). Means (seconds) appear above bars.

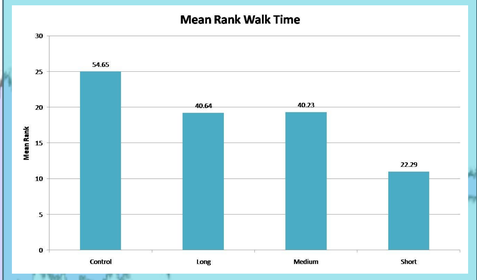


**Figure 6.** Males in the short call group spent the least time in exploratory behavior, again indicating a greater degree of alarm related to the shorter USVs. A Kruskal-Wallis one-way ANOVA did not show a statistically significant effect of call duration ($X^2 = 7.15$, df = 3, $p < 0.0673$). Means (seconds) appear above bars.

## Females Only

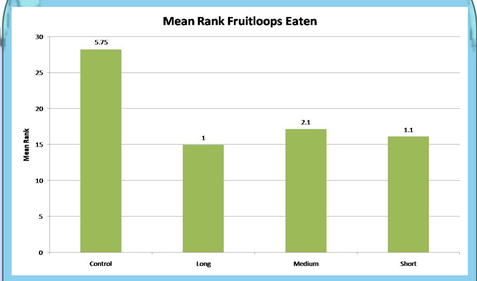


**Figure 7.** Females presented with a USV exhibited less food seeking behavior but showed little variation among the different USV durations. A Kruskal-Wallis one-way ANOVA did not show a statistically significant effect of call duration ($X^2 = 5.53$, df = 3, $p < 0.137$). Means (number of Fruit Loops eaten) appear above bars.

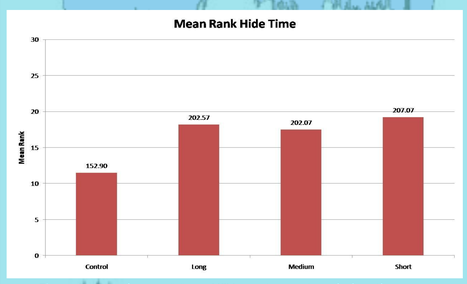


**Figure 8.** Females receiving a USV spent more time hiding than controls, but they varied little in their responses to USVs of different durations. A Kruskal-Wallis one-way ANOVA did not show a statistically significant effect of call duration ($X^2 = 1.79$, df = 3, $p < 0.6165$). Means (seconds) appear above bars.

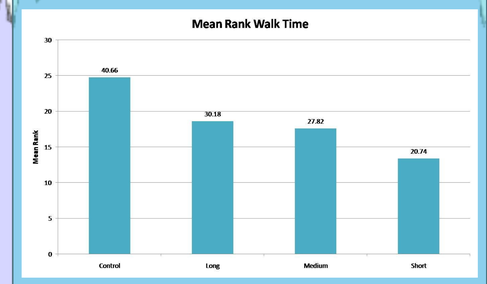


**Figure 9.** Females spent less time in exploratory behavior when they received a USV. Responses were similar across durations, with a slight trend toward a stronger response to short calls. A Kruskal-Wallis one-way ANOVA did not show a statistically significant effect of call duration ($X^2 = 3.94$, df = 3, $p < 0.2682$). Means (seconds) appear above bars.

## Conclusions

- Rats tended to spend more time hiding and less time in exploratory and food seeking behavior when presented with a 22 kHz USV, substantiating the role of these calls as means of communicating threat-related messages.
- Different levels of response between the long, medium and short call groups indicate that call duration is at least one of the characteristics affecting the meaning of 22 kHz USVs.
- USVs of shorter duration appear to convey a greater degree of threat, though this trend may hold only for male rats.
- The more graded response to USV duration seen in males stands in contrast to the "all or nothing" response seen in females and may be related to differences in the roles of the sexes.
- Though consistent trends appeared, many of the results did not achieve statistical significance. The number of subjects, especially in the control group, did not render sufficient statistical power to confirm the relatively subtle differences seen in these behaviors.
- Further experimentation with greater numbers is needed to fully unravel the effect of call duration on the information encoded in 22 kHz USVs and why they are interpreted differently by male and female rats.

## References

Blanchard, R., Agullana, R., McGee, L., Weiss, S., Blanchard, C. (1992) Sex differences in the incidence and sonographic characteristics of anitpredator ultrasonic cries in the laboratory rat. *Journal of Comparative Psychology* 106: 270-277.

Brudzynski, S. (2005) Principals of rat communication: quantitative parameters of ultrasonic calls in rats. *Behavior Genetics* 35: 85-92.

## Acknowledgements

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